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Published in:
Behavioral Ecology

DOI:
10.1093/beheco/arh045

Behavioral Ecology Vol. 15 No. 4: 525–533
DOI: 10.1093/beheco/arh045

Strong evidence for selection for larger brood size in a great tit population

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We measured the selection pressure on brood size in a recently established population of great tits (*Parus major L.*) in the northern Netherlands by manipulating brood size in three years (1995: $n = 51$, 1997: $n = 66$, 1998: $n = 51$), and we estimated fitness consequences in terms of local survival of both offspring and parents. Enlarged broods had highest fitness; the offspring fitness component was positively affected by manipulation and the parental fitness component was unaffected. Parental survival and the probability that parents produced a second clutch were not affected by the treatment. However, parents that had raised enlarged broods produced their second clutch later in the season. Clutch size, brood size, and laying date of birds recaptured in the next breeding season were largely independent of the treatment. We conclude that there is strong evidence for selection for larger brood size and reject the individual optimization hypothesis for this population because the number of young in the nest predicts fitness independently of the manipulation history. *Key words:* brood size, clutch size, fitness, local adaptation, manipulation, *Parus major*. *[Behav Ecol 15:525–533 (2004)]*

With the goal of understanding the adaptive significance of clutch size variation, many studies have manipulated brood size and measured subsequent fitness consequences. The logic is that experimental changes in brood size can be viewed as small mutations. By measuring the fitness effects we can examine whether such a mutation will increase in frequency in the population. The assumptions are: (1) parents react on the manipulation as if the resulting brood size was their own 'decision' (Lessells, 1993), and (2) selection pressure works primarily during the nestling phase and not during egg laying or incubation (Monaghan and Nager, 1997).

Results of brood size manipulation studies showed that both enlarging and reducing brood size may lower fitness, suggesting either that selection pressures stabilize brood size (collared flycatcher *Ficedula albicollis*: Gustafsson and Sutherland, 1988; great tit: Lindén, 1990; Tinbergen and Daan, 1990) or that birds are able to produce a brood size that maximizes their individual fitness (individual optimization of brood size, great tit: Perrins and Moss, 1975; Pettifor et al., 1988; kestrel (*Falco tinnunculus*): Daan et al., 1990; blue tit (*Parus caeruleus*): Pettifor, 1993a,b; great tit: Pettifor et al., 2001; but see Tinbergen and Both, 1999). If individual birds adjust their clutch size to local circumstances, we need an explanation for the fact that different populations of great tits living under different ecological circumstances lay clutches of similar size (van Balen, 1973). Perhaps tits distribute themselves over the available habitat in such a way that resources per pair are relatively constant between habitats (ideal free distribution). Recently, Both et al. (2000) have presented evidence that optimal clutch size is negatively affected by population density and that birds adjust their clutch size accordingly, suggesting that clutch size is adjusted to both food resources and competition for these resources.

An alternative explanation for the small between-population variation in clutch size is that selection may not work at such a fine local scale, causing clutch size to be non-adaptive in some localities (Dhondt et al., 1990; Dias and Blondel, 1996). The observed clutch size is then viewed as a consequence of selection pressures acting over more generations and habitats; gene flow may prevent local adaptation (Kawecki, 1995; McNamara, 1997). In this case we would expect positive, negative, or stabilizing selection in different populations.

Actually, the first thorough brood size experiments led Nur (1984a,b, 1986; but see Pettifor 1993a,b) to conclude that blue tits do not adjust their clutch size to the local situation; larger broods than the population mean led to higher fitness. This idea has won impetus by the work of Verhulst (1995), who showed experimentally that a reduction in brood size enhanced the lifetime reproductive success in an island population of great tits, thus revealing a selection pressure for smaller clutches. On the basis of brood size experiments, Blondel et al. (1998) also found indications for selection for smaller clutches in the Corsican population of blue tits. Both Verhulst (1995) and Blondel et al. (1998) suggested that in these populations gene flow might prevent local adaptation (see also Dias and Blondel, 1996).

In this paper we present the results of a brood size experiment in a recently established population of great tits. We find strong evidence that selection works towards larger broods and then review the brood size manipulation studies in great tits to place these contrasting findings in perspective. Consequences for microevolution are discussed briefly.

METHODS

General

The study area was a young (planted in 1974–1975), mixed deciduous forest in the Lauwersmeer area (The Netherlands, 53° 20′ N, 06° 12′ E). The study was started in 1993 in part of the area that since 1980 had a limited number of nest-boxes (80). From 1994 onwards, around 200 nest-boxes were available in seven plots of differing size (6–106 ha).

Nest-boxes were checked for occupation by great tits at least once a week and the number of eggs, occurrence of incubation, and the number of young were recorded. Laying date was estimated from the assumption that one egg per day was

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Received August 7, 2002; revised May 1, 2003; accepted May 17, 2003.

Table 1
Basic parameters for the manipulated broods

		Manipulation								
		Reduced			Control			Enlarged		
Year		Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
1995	Clutch size	9.76	1.15	17	10.29	0.99	17	9.94	1.3	17
	Number born	9.53	1.12	17	9.53	1.01	17	9.47	1.12	17
	Laying date[a]	26.06	2.68	17	25.76	2.51	17	26.24	2.82	17
1997	Clutch size	9.68	1.43	22	9.86	1.42	22	9.68	1.39	22
	Number born	9.23	1.45	22	9.14	1.13	22	8.73	1.64	22
	Laying date	30.91	5.67	22	30.05	5.18	22	30.68	6.23	22
1998	Clutch size	10.29	1.31	17	9.94	1.09	17	10	1.22	17
	Number born	9.47	1.46	17	8.88	1.83	17	9.12	1.76	17
	Laying date	17	5.62	17	17.35	5.87	17	17	5.87	17

[a] For laying date: 1 = 1 April.

laid. We made daily nest visits around the expected day of hatching. Hatching date is the date on which we recorded the first egg in the clutch to hatch. Adults were captured with spring traps when the young were 7–10 days old (day of hatching = 0) and were ringed, weighed, and measured. At this age the young were ringed as well.

Brood size manipulation

During three years (1995, 1997, 1998), first broods with a similar hatching date (maximum difference of one day), clutch size (maximum difference one egg), and the same number of hatchlings were randomly assigned to one of the groups: reduced, control, or enlarged. Manipulation of brood size was carried out on day 2 after the nestlings hatched (see Sanz and Tinbergen, 1999). On this day three nestlings were transferred quickly between nests to create reduced and enlarged broods of the same age. For each experimental pair of nests, there was a control brood in which brood size was not altered but the nest was subjected to the same disturbance as the reduced and enlarged broods.

The probability of having a second clutch was determined by reading color rings of the females during incubation of the second clutch or by catching the parents with a spring trap in the nest-box during the nestling phase.

Before manipulation the birds did not differ in clutch or brood size between manipulation categories or years (ANOVA, all $p > .1$; Table 1). Laying date did differ between years (ANOVA, $F_{2,163} = 106.0$, $p < .001$) but not within years between manipulation categories (ANOVA, $F_{2,163} = 0.71$, $p > .9$). The group of birds used for the different manipulations can therefore be considered homogeneous within years.

Offspring fitness

We analyzed first brood offspring fitness on a per nest basis in three components: (1) the probability that a brood was successful (at least one chick fledges), (2) the probability that a chick survived from manipulation to fledging from a successful brood, and (3) the probability to recruit when fledged. These components were integrated in the number of recruits per first brood and compared between manipulation categories.

Survival analysis adults

Local survival was estimated on the basis of recaptures of the breeding birds during the next breeding season in the study area. Recapture rate was estimated using the program MARK v 2.1 (White and Burnham, 1999). Experimental birds were included from their experimental year onwards. The animals that had their broods manipulated in more than one year (31 of the 326) were included in the analysis. They were coded as a new individual from the second experiment onwards. Both survival and recapture rates over the first and subsequent winters were estimated as a function of sex, manipulation (together six groups), and time (differences between years). Recapture rate was modeled before survival. Model selection was performed by backward deletion of higher order terms. Because we expected survival effects of manipulation to occur in the first year after manipulation, we specifically tested effects of manipulation on the survivals in these cells (three per group) and estimated the survival in the subsequent years with one sex-specific parameter. The most complicated model included sex, manipulation, and time, but we found no justification to include any of these variables in the model. We used Aikaike's Information Criterion (AIC) to select the most parsimonious model (White and Burnham, 1999).

Fitness calculations

Estimating overall fitness involves both the parental and the offspring components weighed for the age at which reproduction takes place (reproductive value; Fisher, 1930). As no effects of manipulation on reproductive parameters in the subsequent year could be detected, fitness was approximated by the sum of parents and their offspring identified as a breeding bird in the next year. Manipulated broods with unidentified males were excluded, reducing the sample size from 168 to 158. The resulting estimate is slightly biased, because in case of nest failure it is more likely that the identity of the males remains unknown. Because the identity of all females was known, we used the sum of the locally survived females identified in the next year (female parent + female offspring) as a second fitness estimate and compared it with an estimate for the males (male parent + male offspring) using Poisson regression.

In addition, we calculated a formal fitness estimate λ (rate of increase) for the manipulation categories separately and the reproductive values of the 'mutants' reduced and enlarged in a population of control birds ($V[x, x^*]$) to value the fitness differences between them. The reason to calculate λ was to use a well defined fitness estimate that allows correction for recapture probability and enables calculation of $V(x, x^*)$.The estimate of λ was based on P (average annual

Table 2
Effects of manipulation on offspring fitness components

	Manipulation								
	Reduced			Control			Enlarged		
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
Fraction nests fledged	0.95	0.23	56	1	0	56	0.95	0.23	56
Probability of fledging	0.96	0.12	53	0.94	0.11	56	0.91	0.14	53
Probability of recruiting	0.067	0.130	53	0.094	0.116	56	0.096	0.107	53

Given are: fraction nests fledged, probability of fledging for those nests that produced at least one fledgling (number fledged/number after manipulation), and probability of recruiting (number recruits/number fledged).

survival of parents per manipulation category) and F (annual fecundity in terms of recruits from first and second broods divided by two). We assumed age independent survival and fecundity, and no maximum age. In such a simple case, λ can be calculated using the Euler-Lotka equation:

$$1 = F/(\lambda - P)*[1 - (P/\lambda)^n]. \quad (1)$$

For $P < 1$ and $n = \infty$ (the maximum age) this reduces to:

$$\lambda = F + P, \quad (2)$$

where λ is the rate of increase of the population, P is the annual survival, and F is the annual reproduction (see also Charnov and Krebs, 1974).

To see whether a 'mutant' (reduced or enlarged broods) would spread in the natural population (control group) we calculated the reproductive value of the mutant in the population of residents (control group) using:

$$V(x, x^*) = \{F(x)/[\lambda(x^*) - P(x)]\}*\{1 - [P(x)/\lambda(x^*)]^n\}. \quad (3)$$

For $P(x) < \lambda(x^*)$ and $n = \infty$ and using Equation 2 this reduces to:

$$V(x, x^*) = F(x)/[F(x^*) + P(x^*) - P(x)], \quad (4)$$

where x is the manipulation category, $\lambda(x^*)$ is λ of the resident population (control group), $P(x^*)$ and $F(x^*)$ are the annual survival and fecundity of the resident population and $P(x)$ and $F(x)$ are the annual survival and fecundity of the mutant population (reduced or enlarged), respectively. A mutant will spread when $V(x, x^*) > 1$.

Detection of individual optimization

If individual birds maximize fitness by adjusting the clutch size to their local circumstances (individual optimization hypothesis) we expect fitness to peak at original brood or clutch size. Because, in this study population, there were no significant indications of diminishing returns with manipulated brood size, as expected under the individual optimization hypothesis, it is of interest to analyze the fitness effects of manipulation in more detail. This can be done in different ways. We did it by modeling total fitness as a function of the actual number of young after manipulation (Y) and, to allow for diminishing returns, its square (Y^2). Such a model could explain fitness effects of brood size including diminishing returns if only the number of young determined fitness. When individual optimization occurs, one would expect that adding a factor for manipulation (M three levels) and perhaps the interaction M*Y (or M*Y^2) to a Poisson model containing Y and Y^2 should explain additional variance. If adding M would reduce deviance significantly, it means that fitness depends not only on the number of young actually in the nest, but also on the manipulation. This may be the number originally born or some correlate thereof, such as clutch size or local resources. One can also test this idea by including original brood size or clutch size in the analysis. Significant reductions in deviance again point in the direction that some pairs (or their territories) are 'better' than others. This result would be a first step in detecting individual optimization. More specific models are then needed to show that the original clutch size has highest fitness.

Statistics

In cases where the error structure was binomial, logistic regression was used, and Williams correction was used to correct for over-dispersion (Crawley, 1993). For Poisson regression we adjusted the scale parameter according to Crawley (1993, p. 262). Tests were based on likelihood ratios. Interactions were tested but not mentioned in the text when statistically non-significant.

RESULTS

Offspring fitness

Survival until next breeding season

The fraction of nests that fledged at least one chick did not differ between years or between manipulation categories (logistic regression controlled for year, manipulation $\chi^2 = 4.977$, df = 2, $p > .08$, full model deviance 46.699, df 163; Table 2). The control nests tended to be more successful. For the nests that had at least one fledgling ($n = 162$), the probability of fledging was not significantly dependent on manipulation (logistic regression controlled for year, after Williams correction: $\chi^2 = 3.395$, df = 2, $p > .15$, full model deviance 118.21, df 157). The probability of recruiting in the breeding population from fledging onwards was not affected by manipulation (logistic regression controlled for year, after Williams correction: $\chi^2 = 2.545$, df = 2, $p > .3$), nor did it differ between years (logistic regression controlled for manipulation, after Williams correction: $\chi^2 = 2.390$, df = 2, $p > .3$, full model deviance for both models 149.44, df 157).

Poisson regression over the whole material ($n = 168$) showed that the number of recruits per first brood was affected by manipulation ($\chi^2 = 13.44$, df = 2, $p = .001$, scale parameter 1.491, full model deviance 152.15, df 165; Figure 1 and Table 3). The reduced group differed from the control group ($p < .01$) but the enlarged group did not differ significantly from the control group ($p > .2$) when tested pairwise. In a similar analysis using manipulation as a continuous variable ($\chi^2 = 12.46$, df = 1, $p < .001$, scale parameter 1.469, full model deviance 155.7, df 166), no effect of squared

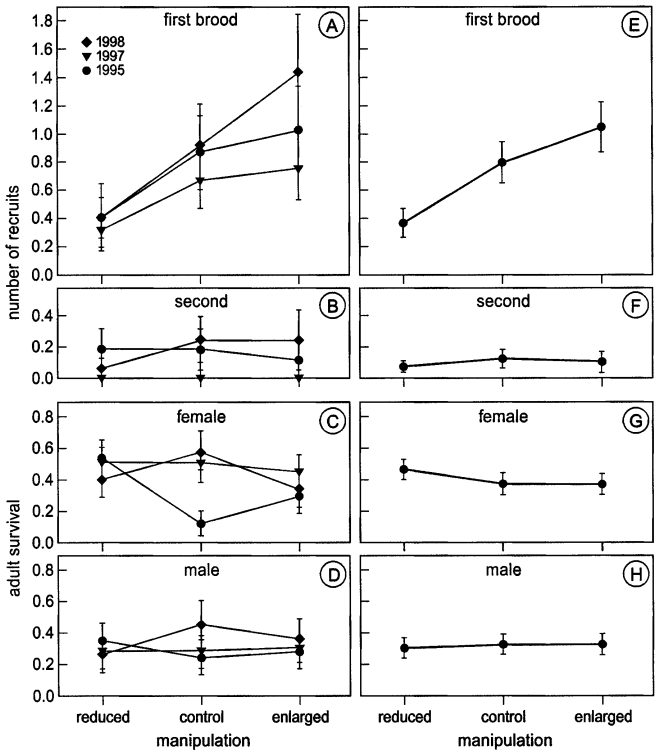

Figure 1
The effect of brood size manipulations on different fitness components. Effects are shown per year separately (A-D: 1995 circles, 1997 triangles, and 1998 diamonds) and combined over the years (E-H). Given are: recruits per first brood (A and E), recruits per second brood (B and F), female survival (C and G), and male survival (D and H). Bars indicate SE. Manipulation effect could only be shown in the recruits per first brood.

manipulation was detectable when added to the significant effect of manipulation ($\chi^2 = 1.195$, df = 1, $p > .2$), nor in interaction with year ($\chi^2 = 1.119$, df = 2, $p > .5$). There are thus no significant indications of diminishing returns with manipulated brood size.

Breeding in the next season
For 60 recruits, data on clutch size, laying date, and offspring born in their first breeding season were available (Table 4). Clutch size and the number of offspring born did not differ with manipulation history (ANOVA, controlled for year, $F_{2,55} = 0.344$, $p > .7$ and $F_{2,55} = 0.474$, $p > .6$, respectively), while differences between years were around significance (ANOVA, controlled for manipulation, $F_{2,55} = 3.153$, $p = .051$ and $F_{2,55} = 3.371$, $p = .042$, respectively). Laying date of the recruits, however, did differ both with manipulation history and year (ANOVA, manipulation effect controlled for year, $F_{2,55} = 3.686$, $p = .031$; ANOVA, year effect controlled for manipulation, $F_{2,55} = 22.005$, $p < .001$), the control group laying the latest and significantly later than the enlarged group (Tukey $p < .05$). We have no explanation for this effect. There were no exceptional differences in body mass, wing length, or tarsus in the nest between the survivors of the control group and the other manipulation categories.

Parental fitness

Second clutches
The probability of starting a second clutch did differ between years (logistic regression, $\chi^2 = 30.0$, df = 2, $p < .001$) but was not different between the manipulation categories ($\chi^2 = 0.27$, df = 2, $p > .8$, residual deviance 177.19, df 157). However, for those pairs that did produce a second clutch, the interval between the first and second clutch depended on manipulation (ANOVA, year effect, $F_{2,49} = 5.814$, $p < .01$, manipulation effect, $F_{2,49} = 5.212$, $p < .01$). Parents with enlarged broods took about one week longer to initiate a second clutch than parents with a reduced brood (Tukey $p < 0.05$). Recruitment of late clutches (second and repeat clutches) did not differ between manipulation categories (Figure 1b).

Parental survival and next year reproduction
The most adequate model that estimated survival and recapture rate of the adults simultaneously was the model with constant recapture and constant survival rate (Table 5). All more complicated models that were tested did not differ significantly from this model (likelihood ratio test all $p > .25$). We conclude that recapture rate was 0.897 (SE = 0.055) and the annual survival rate was 0.413 (SE = 0.0376), both not different between the sexes or with manipulation.

Also, the local survival of both males and females (when estimated simply as the fraction caught next year as a breeding bird) did not differ between years and were not related to manipulation (logistic regression males: $n = 158$, all $p > .7$; females: $n = 168$, all $p > .09$; Figure 1c,d). The large between-year variation in survival reduced the power of this analysis strongly. Clutch size, laying date, and the number of young born in the next year were not affected by the manipulation when controlled for year differences and analyzed for the females ($n = 69$) or for the males ($n = 48$).

Overall fitness

Fitness estimated as the sum of the parents and offspring surviving locally and identified in the next year was affected by manipulation (controlled for year, $\chi^2 = 5.999$, df = 2, $p = .05$, scale parameter 1.23, residual deviance 177.96, df 153). Mean values confirmed the positive selection pressure in this population (reduced = 1.245, control = 1.660, and enlarged = 1.891 locally survived birds per brood; Table 3).

Calculated for females separately, the same trend existed, but the manipulation effect was far from significant (Poisson regression: controlled for year, $\chi^2 = 1.617$, df = 2, $p > .4$, model deviance 122.30, df 167; mean values: reduced = 0.714, control = 0.893, and enlarged = 0.911 locally survived females per brood). This lack of significance relates to the high variation in adult female survival, especially in the control category (see Figure 1c). Recruitment of female offspring was strongly related to manipulation (Poisson regression: $\chi^2 = 12.67$, df = 2, $p < .01$, residual deviance 179.4, df = 165). In males the pattern was consistent and significant (controlled for year, $\chi^2 = 7.574$, df = 2, $p < .05$, scale parameter 1, residual deviance 177.1 df 153; mean values: reduced = 0.528, control = 0.800, and enlarged = 0.982 locally survived males per brood). This pattern is also generated by the significant manipulation effect on the number of male recruits (Poisson regression controlled for year, $\chi^2 = 11.86$, df = 2, $p < .05$, scale parameter 1, residual deviance 156.87, df 153). These results suggest different strength of selection on males and females, but this difference was not statistically significant (controlled for year and manipulation; sex: $\chi^2 = 0.376$, df = 1, $p > .5$, interaction sex and manipulation: $\chi^2 = 1.44$, df = 2, $p > .4$, full model deviance 353.16, df 320).

We calculated λ for each manipulation category on the basis of the data in Table 3. We assumed age-independent survival and reproductive rates and did not correct for dispersal. We used no maximum age but corrected for recapture probability. The fitness estimated in this way for the manipulation categories separately were reduced: $\lambda = 0.686$, control:

Table 3
Fitness components for the three manipulation categories

	Manipulation								
	Reduced			Control			Enlarged		
Fitness Components	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
Offspring first broods									
Clutch size	9.89	1.32	56	10.02	1.20	56	9.86	1.30	56
Number born	9.39	1.34	56	9.18	1.35	56	9.07	1.55	56
Manipulated brood size	6.07	1.55	56	9.18	1.35	56	12.07	1.34	56
Number fledged	5.80	1.97	56	8.61	1.60	56	10.36	3.34	56
Survive to breed (r1)	0.375	0.728	56	0.821	1.064	56	1.071	1.333	56
Offspring later broods									
Probability of late clutch (pr)	0.393	0.49	56	0.339	0.48	56	0.339	0.48	56
Clutch size	7.77	1.57	22	7.58	1.46	19	7.79	0.79	19
Number born	5.55	2.44	22	4.95	2.61	19	5.05	2.88	19
Number fledged	4.77	2.47	22	4.26	2.86	19	4.6	3.06	19
Survive to breed (r2)	0.182	0.501	22	0.368	0.684	19	0.316	0.820	19
Parents									
Survival female (sf)	0.482	0.504	56	0.411	0.496	56	0.375	0.489	56
Survival male (sm)	0.302	0.463	53	0.32	0.471	50	0.327	0.474	55
Offspring + parents									
Breeding next year	1.245	1.125	53	1.66	1.379	50	1.891	1.729	55

Statistics are given per manipulated brood ($n = 56$ per manipulation category) and for the subsequent late broods ($n = 22,19,19$). The survival of female and male parents was based on the birds that were identified (all females [$n = 56$], males 53, 50, and 55, respectively). Survival of offspring and parents was based on the sample where both parents were identified (53, 50, and 55, respectively). For the calculation of λ, F was estimated as 0.5*(r1/c+pr*r2/c), and P as 0.5*(sf/c+sm/c), where c = recapture rate males and females (0.897).

$\lambda = 0.935$, and enlarged $\lambda = 1.048$, indeed suggesting a positive selection pressure on clutch size. The reproductive values of the 'mutants,' reduced and enlarged in the resident population of control birds $V(x, x^*)$, were respectively 0.500 and 1.209, again showing directional selection pressure towards larger broods. This directional selection did not result in an increase in clutch size over the years (Figure 2).

Individual optimization of clutch size?

In this study there were no indications that fitness was maximized at the original clutch size. The total fitness was positively related to Y (the number of young in the nest) only. Y^2 explained no additional variance (Table 6). The fact that M (manipulation), either alone or in interaction with Y or Y^2, did not explain additional variance indicates that fitness depends on the number of young actually in the nest and not on traits of the birds or their resources (Table 6). This conclusion is consistent with the fact that original clutch size or brood size did not explain additional variance. Similar results were obtained in an analysis explaining variation in the number of recruits (Table 6). The fact that we cannot detect diminishing returns with manipulated brood size and that original clutch or brood size did not explain variation in fitness are strong arguments against the occurrence of individual optimization in this population.

DISCUSSION

Interpretation of brood size manipulations

In the Lauwersmeer population we have found strong evidence for positive selection on brood size but no response on this selection in terms of a changing clutch size over the years. There are a number of methodological and biological explanations possible for this lack of response that we will discuss here: (1) we estimated temporal variation in selection on clutch size rather than spatial variation, (2) we use incomplete fitness estimates because of dispersal, (3) there are potential clutch-size related costs in other phases of

Table 4
Effects of treatment on female offspring breeding performance in their first clutch in the next year

	Manipulation								
	Reduced			Control			Enlarged		
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
Clutch size	9.33	1.61	12	8.69	2.47	23	9.00	1.70	25
Laying date	24.0	9.4	12	29.1	10.2	23	21.4	7.2	25
Initial brood size	8.00	1.95	12	8.17	2.57	23	8.28	1.99	25

Table 5
Results of a MARK analysis of survival rate (ϕ) and recapture rate (p) as a constant (.) or a function of sex (s), manipulation (m), and time (t)

Model	QAICc	Delta QAICc	QAICc Weights	Model Likelihood	Num. Par	QDeviance
{ϕ (.) p(.)}	272.20	0.000	0.409	1.000	2	74.96
{ϕ (t) p(.)}	276.44	4.242	0.049	0.120	5	73.10
{ϕ (s) p(.)}	273.34	1.146	0.167	0.564	3	74.08
{ϕ (m) p(.)}	275.22	3.022	0.065	0.221	4	73.92
{ϕ (s+t) p(.)}	277.68	5.488	0.026	0.064	6	72.29
{ϕ (s+m) p(.)}	279.36	7.168	0.011	0.028	6	73.97
{ϕ (t+m) p(.)}	280.46	8.262	0.007	0.016	7	73.00
{ϕ (s+m+t) p(.)}	281.72	9.524	0.004	0.009	8	72.20
{ϕ (s+m+t+s.m+s.t) p(.)}	290.59	18.391	0.000	0.000	13	70.58
{ϕ (s+m+t+t.m+s.t) p(.)}	293.15	20.959	0.000	0.000	15	68.89
{ϕ (s+m+t+t.m+s.m) p(.)}	293.40	21.203	0.000	0.000	15	69.13
{ϕ (s+m+t+t.m+s.m+s.t) p(.)}	297.12	24.926	0.000	0.000	17	68.56
{ϕ (s*m*t) p(.)}	301.42	29.225	0.000	0.000	19	68.52
{ϕ (s*m*t) p(s+m+t+t.m+s.t)}	302.15	29.953	0.000	0.000	23	60.47
{ϕ (s*m*t) p(s+t)}	302.24	30.046	0.000	0.000	22	62.77
{ϕ (s*m*t) p(t)}	302.47	30.276	0.000	0.000	21	65.20
{ϕ (s*m*t) p(s)}	303.03	30.830	0.000	0.000	20	67.95
{ϕ (s*m*t) p(s+m+t)}	303.92	31.725	0.000	0.000	23	62.24
{ϕ (s*m*t) p(s+t)}	304.05	31.852	0.000	0.000	22	64.58
{ϕ (s*m*t) p(s*m*t)}	304.31	32.112	0.000	0.000	24	60.41
{ϕ (s*m*t) p(s+m+t+t.m+s.m+s.t)}	304.31	32.112	0.000	0.000	24	60.41
{ϕ (s*m*t) p(s+m+t+s.m+s.t)}	304.57	32.378	0.000	0.000	24	60.67
{ϕ (s*m*t) p(s+m+t+t.m+s.m)}	304.85	32.654	0.000	0.000	24	60.95
{ϕ (s*m*t) p(s+m)}	305.24	33.046	0.000	0.000	22	65.77

Here the models are arranged by the QAICH. We conclude that the simplest model {ϕ (.) p(.)} is the most adequate model. In this analysis time was coded for the first time step after manipulation only, contrasting estimates of survival directly after manipulation with survival in later years. We corrected for over dispersion by using c- hat = 2.59.

reproduction, and (4) the observed clutch size variation does not represent heritable variation.

(1) With regard to the temporal variation in selection on clutch size, the question is whether the detected differences in fitness are properties of the population under study or merely fluctuations over time, because most of the studies in the different populations have not been performed during the same years (see also Merilä et al., 2001). In our studies the Hoge Veluwe great tit population (1983–1987) and the Lauwersmeer population (1995, 1997, 1998) differed significantly in terms of the effect of manipulation on fitness when tested in one model (interaction population × manipulation, $\chi^2 = 8.433$, df = 2, $p < .05$, residual deviance 682.03, df 510). Only for the year 1998 can a direct comparison be made for the Hoge Veluwe (Visser and Lessells, 2001; recapture probabilities from Bauchau and van Noordwijk, 1995) and the Lauwersmeer (this study). In this year both populations showed a non-significant but positive selection pressure on clutch size (Hoge Veluwe: λ control = 0.83 and λ enlarged 1.12; Lauwersmeer: λ control = 1.20 and λ enlarged = 1.29). For the Hoge Veluwe, this is in contrast to the earlier results (1983–1987), in which selection was stabilizing (Tinbergen and Daan, 1990). Differences in selection between years are probably more a rule than an exception. Because we saw selection for larger brood size in all three years that we measured it in the Lauwersmeer, we believe that this is a population trait rather than a consequence of variation in time. Strictly speaking, longer synchronous data sets from brood size experiments in different areas are needed to come to a firmer conclusion as to what extent the differences found have a spatial or a temporal origin.

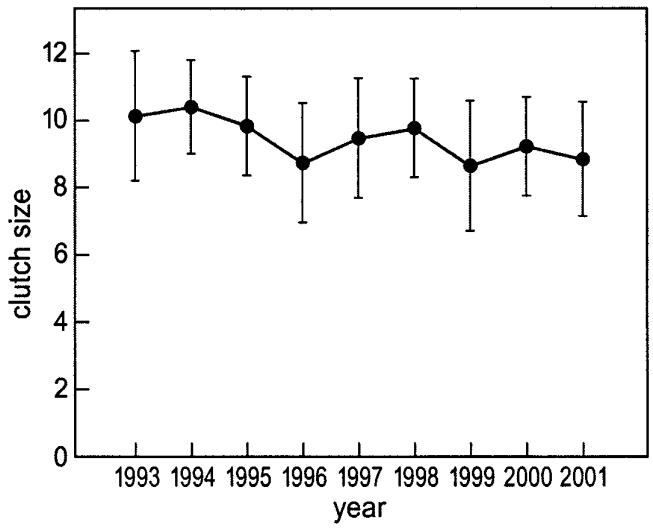

Figure 2
Mean clutch size in the Lauwersmeer did not increase over the years. Bars are SD.

(2) Fitness estimates may be incomplete due to brood size dependent dispersal. Verhulst et al. (1997) showed that the dispersal pattern of young great tits can covary with their nestling mass, and thus it potentially biases fitness estimates that are based on local survival. The Lauwersmeer population differs from most other populations because it consists of a number of wood plots with a maximum distance of 10 km between the plots, surrounded by unsuitable breeding habitat for great tits. In this system, dispersal distance increased with experimental manipulation of brood size (Tinbergen, in

Table 6
Results of Poisson regressions that do not support the individual optimization hypothesis

		(Change in) Deviance	df	*p*
Total fitness, scale parameter 1.250				
Null model:		186.99	157	
Explanatory terms:	Actual brood size (Y)	4.892	1	< 0.05
Full model:		182.1	156	
Rejected terms:	Year	5.353	2	NS
	Actual brood size squared (Y^2)	0.3642	1	NS
	Manipulation (M)	1.102	2	NS
	Clutch size	2.381	1	NS
	Original brood size	0.8402	1	NS
Interactions in model with:				
Y and M:	Y*M	2.505	2	NS
Y, Y^2, M:	Y^2 *M	1.490	2	NS
Number of recruits, scale parameter 1.482				
Null model:		179.59	167	
Explanatory terms:	Actual brood size (Y)	13.17	1	< 0.001
	Year	6.083	2	< 0.05
Full model:		160.34	164	
Rejected terms:	Actual brood size squared (Y^2)	0.2215	1	NS
	Manipulation (M)	2.330	2	NS
	Clutch size	1.533	1	NS
	Original brood size	0.7889	1	NS
Interactions in model with:				
Y, M and Year:	Y*M	0.332	2	NS
Y, Y^2, M and Year:	Y^2 *M	0.612	2	NS

Analyses of both total fitness (the sum of the parents and their offspring recaptured next year) and the number of recruits (offspring from parents of a manipulated first brood from all broods in the manipulation year recaptured next year) are given. Actual brood size (young after manipulation or Y) explains the variation in fitness estimates. NS: not significant.

preparation). Such dispersal may reduce the chance to find surviving offspring locally with increasing brood size. This could lead to underestimation of the positive selection pressure in the Lauwersmeer. If the same effect exists in other populations, it may seriously affect our judgment of local adaptation of brood size, and further analysis for other populations is also required.

(3) Selection may not act in the nestling phase alone; it has important components during other phases, such as egg laying or incubation. Recently, evidence has accumulated that the egg laying and incubation periods cannot be neglected when estimating selection pressures on clutch size (Monaghan and Nager, 1997). The question arises: what we can conclude about selection pressures on *clutch size* on the basis of *brood size* manipulation studies? Visser and Lessels (2001) estimated (in the Hoge Veluwe population) the fitness loss due to laying and incubation of extra eggs (comparison of their 'full costs' treatment with their 'free chicks' treatment) to be around 0.3 female units for one to two eggs. If the cost of egg laying is indeed so high, it could also seriously change our view on selection acting on clutch size in tits.

(4) The observed clutch size variation did not represent heritable variation. When variation in clutch size is associated with variation in fitness, and this variation has a heritable component, we expect natural selection to occur (Endler, 1986). Using our experimental approach we showed that brood size variation was associated with fitness variation, and the general idea is that clutch size in great tits has heritable variation. When calculated for the whole Lauwersmeer population, the heritability of clutch size was indeed positive and significant (daughter-mother regression: $h^2 = 0.272$, SE = 0.118, $p = .02$, $n = 242$). However, when controlling for plot for both daughter and mother, this value remained positive but became insignificant ($h^2 = 0.166$, SE = 0.122, $p > .15$), suggesting that a considerable part of the variation was induced by the environment. The lack of response to the selection for larger clutches may thus be caused by a lack of (or a weak) heritable variation in clutch size in this population. Such an explanation for the lack of response to selection was formulated earlier by Price et al. (1988) and was recently illustrated by the work of Kruuk et al. (2002) on antler size in red deer. Variation in antler size was associated with lifetime breeding success and had a heritable component, but fitness differences seem to be largely associated with the environmentally caused variation in antler size.

Comparison to other studies

Only when we assume that the above-mentioned biases in the fitness measurements can be neglected is it useful to discuss the results of the current study in relation to others. We compiled the outcome of brood size manipulation studies in great tits over the last few decades to see what the conclusions of these experiments were regarding the selection on brood size (Table 7) within this well studied species. Fitness effects as judged by the authors are given for parents and brood separately. Their judgment of the overall effect is also given. For the great tit, there are nine studies based on data from five woods.

The great tit data of Wytham wood (UK) have been analyzed by different people using basically the same data set. In their brood size manipulation study, Perrins and Moss (1975) signaled that the most productive brood size estimated from experimental variation was close to the original brood size, in contrast to the larger most productive brood size calculated on the basis of natural variation in brood size. They

Table 7
Overview of the fitness effects of brood size manipulations in the great tit

Study Area	Authors	Selection			Years
		Brood Component	Parental Component	Brood Size Overall	
Wytham	Perrins and Moss, 1970	positive	?	?	1958–1970
Wytham	Boice and Perrins, 1987	stabilizing[a]	neutral	stabilizing[a]	1960–1983
Wytham	Pettifor et al., 1988	stabilizing	neutral	stabilizing	1959–1963, 1977–1980
Wytham	Pettifor et al., 2001	stabilizing	neutral	stabilizing	1959–1964, 1977–1980
Gotland	Lindén, 1990	stabilizing	neutral	stabilizing	1985–1989
Hoge Veluwe	Tinbergen and Daan, 1990	stabilizing	negative	stabilizing	1983–1987
Lauwersmeer	This study	positive	neutral	positive	1995–1998
Vlieland	Verhulst, 1995*	negative	negative	negative	1989–1991
Hoge Veluwe	Visser and Lessells, 2001**	negative	positive	positive	1998–1999

The direction of selection is given for the brood and the parental component separately, and for the combination of the two. * Only broods reduced; ** only broods enlarged.

[a] If including annual variation.

suggested that this contrast might be a result of the fact that tits adjust their clutch size to produce their own most productive brood size in terms of fitness (later called the individual optimization hypothesis, Pettifor et al., 1988). Boyce and Perrins (1987) analyzed the effect of a fluctuating environment on the geometric mean fitness of the most productive brood size in the same data set extended with new years. This analysis led to the different view that also in the case of artificial variation in brood size, the most productive brood size was larger than the original brood size. Their point was that bad seasons affected larger broods relatively more, reducing optimal brood size in terms of geometric mean fitness below the average most productive brood size. No individual adjustment of clutch size was needed to explain the observed patterns. Pettifor et al. (1988, 2001) did a reanalysis of the same data set but used different statistical models. Their analysis, more consistent with the view in the paper of Perrins and Moss (1975), led to the conclusion that stabilizing selection on individual brood size (individual optimization of clutch size) is the rule in the Wytham great tit population. Apparently, for Wytham the conclusion depends on the analysis and/or the fitness measure used. Because analyses are complicated and different fitness measures have been used, comparison between studies es hampered to some extent. Pettifor et al. (2001) clearly favor the individual optimization hypothesis as an explanation for the observed patterns, but they did not discuss why Boyce and Perrins (1987) reached a different conclusion using the same data.

Experimental studies on Gotland (Lindén, 1990) and on the Hoge Veluwe (Tinbergen and Daan, 1990) confirmed Pettifor's view. These authors also found evidence for stabilizing selection on brood size in line with the individual optimization hypothesis. However, Verhulst (1995) found a population with strong selection for smaller broods. He reduced brood size in an island population of great tits and showed that this reduction led to a fitness increase. Together with the results of the current study, we think that the claim of Pettifor et al. (2001), that brood size experiments are generally in favor of the individual optimization hypothesis, does not hold.

The fact that there are populations with selection for larger (this study) and for smaller (Verhulst, 1995) broods is consistent with the gene flow hypothesis (Dhondt et al., 1990; Dias and Blondel, 1996). As a matter of fact, Perrins and Moss (1975) also suggested that clutch size could be a product of differing selection in different habitats. However, other explanations can be given for a lack of microevolution under directional selection in natural populations, as recently reviewed by Merilä et al. (2001).

The outcome of our work urges us to formulate how ideas of individual optimization blend with ideas on microevolution. Suppose that in a population with perfect individual optimization of clutch size, a heritable component of clutch size exists. If larger clutches have higher fitness (Perrins and Moss, 1975; Tinbergen and Daan, 1990), we would expect directional selection towards larger clutches and need an explanation why this generally does not happen.

If individual optimization of clutch size were perfect, we would not expect a heritable component in the variation in clutch size. The flexible phenotype of the omniscient bird would produce exactly the number of eggs at a time that maximizes fitness. In this view, variation in clutch size would be environmentally determined and we expect competition for the better places, in concert with density dependent effects, to govern clutch size variation (Both et al., 2000).

In this paper we showed that flexible phenotypes are not always perfect. Great tits laid clutches that were not locally adapted. This is in line with the fact that the environment is not perfectly predictable. Even if individuals can adjust their reproduction on the basis of a learning process, adjustment will lag behind. On a longer time scale, adaptation may take place via microevolution. In this process, natural selection will shape the genetic architecture, and whether or not we will find locally adapted traits will depend on the interactions between selection, the genetic architecture, and spatial and temporal variation in the environment.

We thank J. Willems, S. Krap, and H. Hut from 'Staatsbosbeheer' and J. H. Janssen, E. K. Hansen, and G. Altena from the 'Koninklijke Landmacht' for their permission to work in the Lauwersmeer area. We are grateful for the help of many students. We profited a lot from critical reading of the paper by C. Both, R. H. Drent, T. Piersma and J. Komdeur. J.J.S. was supported by a postdoctoral grant from the Ministerio Español de Educación y Cultura and by a contract from projects PB94-0070-C02-01 and PB97-1233-C02-01.

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